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#### **Declarations under Rule 4.17:**

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(54) Title: FLUORESCENCE TECHNIQUE FOR ON-LINE MONITORING OF STATE OF HYDROGEN-PRODUCING MI-CROORGANISMS

(57) Abstract: In situ fluorescence method to monitor state of sulfur-deprived algal culture's ability to produce H2 under sulfur depletion, comprising: a) providing sulfur-deprived algal culture; b) illuminating culture; c) measuring onset of H2 percentage in produced gas phase at multiple times to ascertain point immediately after anerobiosis to obtain H2 data as function of time; and d) determining any abrupt change in three in situ fluorescence parameters; i) increase in Ft (steady-state level of chlorophyll fluorescence in light adapted cells); ii) decrease in  $F_{m'}$  (maximal saturating light induced fluorescence level in light adapted cells); and iii) decrease in  $\Delta F/F_m' = (F_m' - F_t)/F_m'$  (calculated photochemical activity of photosystem II (PSII) signaling full reduction of plastoquinone pool between PSII and PSI, which indicates start of anaerobic conditions that induces synthesis of hydrogenase enzyme for subsequent H<sub>2</sub> production that signal oxidation of plastoquinone pool asmain factor to regulate H<sub>2</sub> under sulfur depletion.